129. (New) The method of claim 1, further comprising identifying a subject in need of a mucosal immune response.

130. (New) A method for inducing a mucosal immune response, comprising:

administering to a mucosal surface of a subject an effective amount for inducing a mucosal immune response of an oligonucleotide at least 8 nucleotides in length, formulated for ocular administration, rectal administration, vaginal administration, intranasal administration or inhalation, and having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein $X_1$, $X_2$, $X_3$, and $X_4$ are nucleotides, and

exposing the subject to an antigen to induce the mucosal immune response, and wherein the antigen is not encoded in a nucleic acid vector.

## Remarks

*Specification Amendments:*

Specification amendment 1 corrects the SEQ ID NO: designation for oligonucleotide number 1982, and includes the SEQ ID NO: designation for oligonucleotide number 1826. Support for these amendments can be found in the Examples and the Paper Sequence Listing. (See Examples, page 57, lines 10-11.)

Specification amendment 2 corrects a typographical error by replacing "descried" with "described."

Specification amendment 3 corrects a typographical error by replacing "he" with "the."

Specification amendment 4 corrects a typographical error. The doses to be administered to subjects should be expressed in μg rather than mg. Support for this amendment can be found in the Examples which teach administration of 1-10 μg of antigen or oligonucleotide per murine subject. (See, for example, Table 2.) Further support can be found in the provisional priority document that teaches administration of 1-10 μg of HBV S antigen either alone or together with 1-10 μg of CpG oligonucleotide. (See U.S. Provisional Patent Application 60/086,393.)

Specification amendment 5 corrects typographical errors relating to μl or μg. Support for these amendments can be found in the Figures as well as in the priority document. (See U.S. Provisional Patent Application 60/086,393.)

No new matter has been added.

*Claim Amendments:*

Claims 1-28 and 125-130 are currently pending.

Claim 1 has been amended to recite an oligonucleotide length of at least 8 nucleotides. Support for this amendment can be found on page 8, lines 17-18. The claim has been further amended to recite that the subject is one in need of a mucosal immune response. Support for this amendment can be found throughout the specification and specifically on page 7, lines 19-23, page 16, lines 6-19 and 30, page 17, lines 1-4, page 18, lines 4-10, page 27, lines 27-30 and page 28, lines 1-5.

Claim 20 has been amended to remove the term "muticellular organisms such as".

Claim 27 has been amended to recite a "boost of the oligonucleotide". Support for this amendment can be found on page 26, lines 28-32.

New claims 125-130 have been added. Support for these new claims can be found throughout the specification and particularly on page 5, lines 17-24 (claim 125); page 4, lines 22-23 and page 8, lines 10-11 (claim 126); page 4, lines 22-23, and page 9, line 18 (claim 127); page 9, lines 6-10, page 45, lines 15-22, page 53, lines 3-10 and 27-29, and page 57, lines 17-24 (claims 128 and 130); and page 7, lines 19-23, page 16, lines 6-19 and 30, page 17, lines 1-4, page 18, lines 4-10, page 27, lines 27-30 and page 28, lines 1-5 (claim 129).

Applicants reserve the right to pursue the subject matter of the originally filed claims in a continuing application.

No new matter has been added.

**The Claimed Invention**

The claimed invention relates to a method for inducing a mucosal immune response. The ability of CpG oligonucleotides to induce a mucosal immune response when administered to a mucosal surface, and thus to function as a mucosal adjuvant, had not been discovered prior to the present invention. The claimed methods involve administering to a subject in need of a mucosal immune response a CpG oligonucleotide that is at least 8 nucleotides in length at a mucosal surface and exposing the subject to an antigen. Antigen exposure can be active (via direct administration) or passive (via random environmental contact). The oligonucleotide is administered in an effective amount to induce a mucosal immune response at local or remote mucosal sites (i.e., that amount necessary to cause the development of IgA in response to an antigen following antigen exposure). The method can further comprise, in various embodiments, administration of a non-oligonucleotide mucosal adjuvant, a cytokine, or a B-7 costimulatory molecule. Other claimed methods include administering the oligonucleotide in a formulation for particular mucosal delivery routes (e.g., ocular administration, rectal administration, vaginal administration, intranasal administration, and inhalation).

**Rejection under 35 U.S.C. 112, first paragraph**

Claims 1-10, 12 and 14-22 and 24-28 are rejected under 35 U.S.C. 112, first paragraph because the specification "does not reasonably provide enablement for methods of administering any CpT (sic) motif containing oligonucleotide of less than 8 nucleotide residues including the elected species of 5' $X_1X_2CGX_3X_4$ 3' wherein $X_1$ is G, $X_2$ is T, $X_3$ is T, and $X_4$ is T for inducing a mucosal immunity to a recombinant peptide/polypeptide antigen within the context of therapeutic applications." The Examiner acknowledges that the specification enables a method for "inducing a mucosal immune response, comprising: administering to a mucosal surface of a subject an effective amount for inducing a mucosal immune response of an oligonucleotide having a length of at least 8 nucleotide residues and comprising CpG motif containing oligonucleotide including the elected species of 5' $X_1X_2CGX_3X_4$ 3' wherein $X_1$ is G, $X_2$ is T, $X_3$ is T, and $X_4$ is T; and administering to the subject an antigen not encoded in a nucleic acid vector to the subject to induce a mucosal immune response." In support of his rejection, the Examiner raises three points. First, the Examiner states that claim 1 and claims dependent therefrom are not enabled because these claims fail to affirmatively recite a step of administering an antigen to the claimed subject. Second, the Examiner states that the claims are not enabled to the extent that they recite oligonucleotides of less than 8 nucleotide residues. Third, the Examiner states that the claim is enabled only for oligonucleotides that comprise the 5' GTCGTT 3' motif.

Applicants respectfully traverse the Examiner's first point. Claim 1 as currently pending recites a method for inducing a mucosal immune response comprising administering to a mucosal surface of a subject an effective amount for inducing a mucosal immune response of an oligonucleotide having a particular sequence and length, and exposing the subject to an antigen that is not encoded in a nucleic acid vector. Accordingly, the claim does affirmatively recite a step of exposing the subject to an antigen. The term "exposing the subject to an antigen" intends to embrace both passive and active exposure to the antigen, as evidenced by dependent claims 2 and 10 and as described in the specification on page 27, lines 14-26. As a result, although the Examiner suggests that the claim be amended to affirmatively recite a step of "administering an antigen to the subject," the claim need not be narrowed to this extent. The specification teaches that the subject can be exposed to the antigen by direct administration by any route including but not limited to oral, intranasal and intratracheal routes, or by entry of a foreign pathogen into the body as an example of passive exposure.

As to the Examiner's second point, Applicants have amended claim 1 to recite that the oligonucleotide is at least 8 nucleotides in length.

Applicants respectfully traverse the Examiner's third point. The prior art references cited by the Examiner (i.e., WO96/02555 and USP 6,218,371) evidence the ability of a range of CpG oligonucleotides

having various CpG motifs to function as immunostimulants. The Examiner has provided no basis to support his assertion that other CpG motifs would not similarly induce mucosal immunity.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 112, first paragraph.

**Rejection under 35 U.S.C. 112, second paragraph**

Claims 1, 2, 4, 8, 10, 20 and 26-28 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Claims 1, 2 and 10 are indefinite, according to the Examiner, because of the recitation of "exposing" and "exposing passively" because it is not apparent what materials or steps are required to achieve the exposing. As stated above, the specification defines the term "exposed to" as embracing the active step of contacting the subject with an antigen or the passive exposure of the subject to the antigen. (See page 27, lines 14-16.) Active exposure means that the antigen is administered directly and deliberately to a subject (e.g., by oral, intranasal, intratracheal, vaginally, or rectal delivery). (See page 27, lines 16-20.) Passive exposure means that the subject is exposed to the antigen randomly because of the environment the subject is in. As example of passive exposure is the entry of a foreign pathogen into the body of the subject or the development of a tumor cell expressing a foreign antigen on its surface. Accordingly, the terms "exposing" and "exposing passively" are definite in view of the specification, as are the claims that recite these terms.

Claims 2, 8 and 26-28 are indefinite, according to the Examiner, because it is not apparent as to where the administering occurs. Applicants believe the rejection is directed to claims 2, 8 and 27-28 because claim 26 does not include a step of administering. The claims as pending relate to administration of an oligonucleotide to a subject at a mucosal surface and exposure of a subject to an antigen. The specification teaches various mucosal administration routes including oral, intranasal, intratracheal, inhalation, ocular, vaginal and rectal. The antigen exposure can occur at a variety of tissues or surfaces including but not limited to mucosal surfaces. The claims also relate to administration of cytokines, co-stimulatory molecules, non-oligonucleotide mucosal adjuvants, and boost doses of oligonucleotides, any or all of which may occur at mucosal surfaces. Accordingly, the term "administering" is definite in view of the specification, as are the claims that recite this term.

Claim 21 is indefinite, according to the Examiner, because of the recitation of "such as." Applicants have amended claim 20 to delete the phrase "muticellular organisms such as" and to recite parasites as antigens. Accordingly, claim 21 is definite in view of this amendment.

Claim 26 is indefinite, according to the Examiner, because the intended meaning of "remote" is not apparent. The specification teaches that a remote site is a mucosal tissue that is located in a different region of the body than the mucosal tissue to which the CpG oligonucleotide has been administered. As an example, the specification teaches that if a CpG oligonucleotide is administered intranasally, then a remote site would be a mucosal lining of the gut. (See page 49, lines 7-10). Accordingly, the term "remote" is definite in view of the specification.

Claim 27 is indefinite, according to the Examiner, because it is not apparent whether "a boost oligonucleotide" refers to the previously administered oligo or other oligonucleotides. Applicants have amended claim 27 to recite "a boost of the oligonucleotide." Accordingly, claim 27 is definite in view of this amendment.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejections under 35 U.S.C. § 112, second paragraph.

**Rejection under 35 U.S.C. 102(b)**

*In view of Krieg WO96/02555*

Claims 1-7, 10, 12, 14-22 and 26-27 are rejected under 35 U.S.C. 102(b) as being anticipated by Krieg (WO96/02555), as evidenced by Moldoveanu et al., Sato et al., McCluskie et al., and Mestecky et al.

According to the Examiner, Krieg teaches concepts identical to those of the instant disclosure because the reference teaches oral administration of the oligonucleotide and/or antigen, CpG motifs including 5' GTCpGTT 3', and IL-6 production following injection of a CpG motif containing oligonucleotide to mice. The Examiner relies on the remaining references as factual evidence that CpG oligonucleotides, when administered to the mucosal surface of a subject, generate a mucosal immunity and that local production of IL-6 at a mucosal surface stimulates production of IgA antibodies. The Examiner concludes that the method of Krieg would inherently generate the production of mucosal immunity. Applicants respectfully traverse the rejection for the reasons stated below.

In order for a reference to anticipate, it must disclose each and every limitation of the claimed invention. The Krieg reference does not disclose several limitations of the pending claims. First, the Krieg reference does not teach administration of a CpG oligonucleotide to a subject in need of a mucosal immune response. (See claim 1 and claims dependent thereon.) The reference does not teach that the methods described therein are capable of inducing a mucosal immune response (e.g., as demonstrated by the production of IgA). As a result, the reference cannot teach that appropriate subjects are those in need of a mucosal immune response. Second, the reference does not specifically recite mucosal administration of a CpG oligonucleotide by a route other than oral delivery. (See claim 1 and claims dependent thereon.)

Third, the reference does not specifically recite formulation of a CpG oligonucleotide for ocular administration, rectal administration, vaginal administration, intranasal administration or inhalation. (See claims 128 and 130.)

The remaining references are moot as to their evidentiary weight because the Krieg reference does not anticipate the claimed invention.

*In view of Krieg USP 6,218,371*

Claims 1-10, 12, 14-22 and 24-28 are rejected under 35 U.S.C. 102(e) as being anticipated by Krieg et al. (USP 6,218,371), as evidenced by Moldoveanu et al., Sato et al., McCluskie et al., and Mestecky et al. According to the Examiner, Krieg et al. teaches oral and nasal administration of an oligonucleotide and/or an antigen, CpG motifs including 5' GTCpGTT 3', the use of a colloidal dispersion system, the use of a cytokine including B-7 as an adjuvant in combination with a CpG containing oligonucleotide of at least 8 nucleotides, and IL-6 production. The Examiner relies on the remaining references as factual evidence that CpG motifs when administered to the mucosal surface of a subject generate a mucosal immunity, and that local production of IL-6 at the mucosal surface stimulates production of IgA antibodies. The Examiner concludes that Krieg et al. would inherently generate mucosal immunity.

Applicants submit herewith a Declaration under 37 C.F.R. 1.131 stating that the claimed invention was conceived of and reduced to practice prior to the effective filing date of USP 6,218,371. The Declaration is submitted herewith as Appendix C (unsigned). Applicants intend to submit a signed version of the Declaration as well as any corroborative support that is deemed to be required by the Examiner. In view of the Declaration, USP 6,218,371 is no longer prior art to the instant application.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejections under 35 U.S.C. § 102(b) and 102(e).

**Rejection under 35 U.S.C. 103(a)**

Claims 1-10, 12, 14-22 and 24-28 are rejected under 35 U.S.C. 103(a) as being unpatentable over either Krieg (WO96/02555) or Krieg et al. (USP 6,218,371), taken with Krieg et al. (*Trends in Microbiology* 6(1):23-27, 1998) as evidenced by Moldoveanu et al., Sato et al., McCluskie et al. and Mestecky et al. According to the Examiner, although the Krieg references do not teach the use of Th2 response inducing adjuvants (i.e., alum), Krieg et al. (*Trends in Microbiology*) teaches that alum is effective as a Th2 response inducing adjuvant. The Examiner concludes that it would have been obvious for one of ordinary skill to have used alum in the immunization methods of either of the Krieg references.

The Examiner further states that it is well established that mucosal immunity is the same as a Th2 response. Applicants respectfully traverse the rejection for the reasons stated below.

Applicants traverse the Examiner's assertion that it is well established that mucosal immunity is the same as a Th2 response. The data of the instant invention clearly rebut this assertion by demonstrating that CpG oligonucleotides induce a mucosal immune response of a Th1 type. The instant specification teaches that Cholera toxin is a mucosal adjuvant that induces a Th2 immune response. Accordingly, a mucosal immune response is not synonymous with a Th2 immune response.

Applicants argue above that the Krieg reference (WO96/02555) does not anticipate the claimed invention, as presently claimed. Applicants also assert that their date of invention is earlier than the effective filing date of Krieg et al. USP 6,218,371, thereby removing this reference as prior art. The secondary reference (Krieg et al. Trends in Microbiology) does not provide the deficiencies of the primary Krieg reference (WO96/02555). In particular, the secondary reference does not teach that mucosal immunity can be achieved upon mucosal administration of CpG oligonucleotides. Accordingly, the reference cannot teach administration of CpG oligonucleotides to a subject in need of a mucosal immune response or administration of a CpG oligonucleotide in an amount effective to induce a mucosal immune response (as evidenced by the production of IgA). The secondary reference also does not teach formulation of CpG oligonucleotides for ocular administration, vaginal administration, rectal administration, intranasal administration or inhalation. The combination of the primary Krieg reference with the secondary Krieg reference does not render obvious the claimed invention.

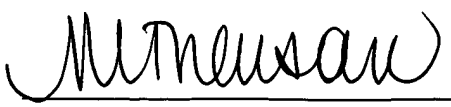
In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 103(a).

**Summary**

Applicants believe that each of the pending claims is in condition for allowance. Applicants respectfully request that the Examiner telephone Applicants' agent in the event that the claims are not found to be in condition for allowance.

If the Examiner has any questions and believes that a telephone conference with Applicants' agent would prove helpful in expediting the prosecution of this application, the Examiner is urged to call the undersigned at (617) 720-3500 (extension 266).

Respectfully submitted,



Maria A. Trevisan, Reg. No. 48,207

579463.2

Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, MA 02210-2211
(617) 720-3500

Docket No. C1040/7006 (HCL/MAT)
Date: December 20, 2001
**x12/20/01**

## Appendix A

## Marked-Up Specification and Claims

Please re-write the two paragraphs starting on page 10, line 24, as follows:

Figure 7 is a graph depicting the effect of different adjuvants on total IgG titers of anti-HBs, wherein BALB/c mice were immunized by IN inhalation with HBsAg (1 μg) without or in combination with Cholera toxin (CT), *Escherichia coli* heat-labile enterotoxin (LT), the B subunit of Cholera toxin (CTB), a detoxified mutant of *Escherichia coli* heat-labile enterotoxin (LTK63), CpG oligonucleotide (motif #1826, SEQ ID NO. 90) or non-CpG control oligonucleotide (motif #1982, SEQ ID NO. [90] 91), as adjuvants (1, 10 or 500 μg). In groups which responded, all mice gave titers > 10, except in the case of 10 μg LT where only 1/5 mice responded.

Figure 8 is a bar graph depicting the effect of different prime/boost strategies on total IgG titers of anti-HBs, wherein BALB/c mice were immunized: (i) by IM injection with HBsAg (1 μg) in combination with alum plus CpG oligonucleotide (motif #1826, SEQ ID NO. 90) and boosted at 4 weeks as prime, or by IN inhalation with HBsAg (1 μg) without or in combination with Cholera toxin (CT) and /or CpG oligonucleotide (motif #1826, SEQ ID NO. 90); or (ii) by IN inhalation with HBsAg (1 μg) without or in combination with Cholera toxin (CT) and /or CpG oligonucleotide (motif #1826, SEQ ID NO. 90) and boosted at 4 weeks as prime or by IM injection with HBsAg (1 μg) in combination with alum plus CpG oligonucleotide (motif #1826, SEQ ID NO. 90). Numbers at the top of each bar represent the IgGa/IgG1 ratio.

Please re-write the paragraph starting on page 30, line 12, as follows:

Other medically relevant microorganisms have been [descried] described extensively in the literature, e.g., see C.G.A Thomas, *Medical Microbiology*, Bailliere Tindall, Great Britain 1983, the entire contents of which is hereby incorporated by reference.

Please re-write the paragraph starting on page 40, line 24, as follows:

The invention also utilizes polynucleotides encoding the antigenic polypeptides. It is envisioned that the antigen may be delivered to the subject in a nucleic acid molecule which encodes for the antigen such that the antigen must be expressed *in vivo*. Such antigens delivered to [he] the subject in a nucleic acid vector are referred to as "antigens encoded by a nucleic acid vector." The nucleic acid encoding the antigen is operatively linked to a gene expression sequence which directs the expression of the antigen nucleic acid within a eukaryotic cell. The "gene expression sequence" is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the antigen nucleic acid to which it is operatively linked. The gene

expression sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPTR), adenosine deaminase, pyruvate kinase, β-actin promoter and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the cytomegalovirus (CMV), simian virus (e.g., SV40), papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of Moloney leukemia virus and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

Please re-write the paragraph starting on page 50, line 28, as follows:

Subject doses of the compounds described herein typically range from about 80 [mg] μg/day to 16,000 [mg] μg/day, more typically from about 800 [mg] μg/day to 8000 [mg] μg/day, and most typically from about 800 [mg] μg/day to 4000 [mg] μg/day. Stated in terms of subject body weight, typical dosages range from about 1 to 200 [mg] μg/kg/day, more typically from about 10 to 100 [mg] μg/kg/day, and most typically from about 10 to 50 [mg] μg/kg/day. Stated in terms of subject body surface areas, typical dosages range from about 40 to 8000 [mg] $\mu g/m^2$/day, more typically from about 400 to 4000 [mg] $\mu g/m^2$/day, and most typically from about 400 to 2000 [mg] $\mu g/m^2$/day.

Please re-write the paragraphs starting on page 57, line 16, as follows:

**2. Mucosal Immunization**

Each animal was immunized with 1 or 10 [Fg] ug plasma-derived HBV S protein (HBsAg, ad subtype, Genzyme Diagnostics, San Carlos, CA), which was administered alone or in combination with 1 or 10 μg of CT or LT or derivative of them and/or CpG oligonucleotide #1826. The derivatives of CT were the B subunit of CT (CTB). The detoxified derivatives of LT were all produced by genetic mutations that affected the A subunit or enzymatic activity and included LTK63. All vaccines were delivered in a total volume of 150 μl, which was applied as droplets directly over both external nares of lightly anaesthetized mice. Some mice were boosted in the identical manner at 8 weeks after prime. All experimental groups contained 5 or 10 mice.

**3. Collection of samples**

Plasma: Plasma was recovered from mice at various times after immunization (1, 2, 4 and 8 wk post-prime and 1, 2 and 4 wk post-boost) by retro-orbital bleeding and stored at -20°C until assayed.

*Fecal pellets*: Fecal pellets were collected from mice at various times after immunization (1, 2, 4 and 8 wk post-prime and 1, 2 and 4 wk post-boost). Mice were isolated in individual cages without bedding for a 24 hr period, following which fecal pellets were collected and weighed into 0.1 mg aliquots. One ml TBS (0.05 M Tris-HCl, 0.15 M NaCl, pH 7.5) and 0.1 [Fg] µg sodium azide (Sigma) were added per 0.1 mg of fecal material. Samples were allowed to rehydrate for 30 min at RT, then were centrifuged at 6000 rpm for 15 min. to remove fecal debris and supernatants were collected and stored at –20[E] ° C until assayed for S-IgA by ELISA.

Lung washes: Lung washes were carried out on mice 4 wk after primary immunization or boost. A 0.33 cc Insulin syringe with a 29G1/2 needle attached (Becton [Dickenson] Dickinson, Franklin Lakes, NJ) was used for carrying out lung washes. One ml TBS was drawn into the syringe and a length of polyethylene (PE) tubing that was 1 cm longer than the needle was attached (PE20, ID = 0.38 mm, Becton Dickinson). The mouse was killed by anesthetic overdose and the trachea was immediately exposed through an anterior midline incision made using fine-tipped surgical scissors (Fine Science Tools Inc., North Vancouver, BC). A small incision was then made in the trachea and a clamp (Fine Science Tools Inc., North Vancouver, BC) was placed above it. The PE tubing was passed a few mm down the trachea through the incision and a second clamp was placed just below the incision to hold the PE tubing in place in the trachea. The TBS solution was slowly instilled in the lungs then withdrawn three times (80% recovery expected). Recovered samples were centrifuge at 13,000 rpm for 7 min., and the supernatants were collected and stored at –20[E] ° C until assayed by ELISA.

**4. Evaluation of immune responses**

*Systemic humoral response*: HBsAg-specific antibodies (anti-HBs) in the mouse plasma were detected and quantified by end-point dilution ELISA assay (in triplicate) for individual animals as described previously (Davis *et al.*, 1998). Briefly, 96-well polystyrene plates (Corning) coated overnight (RT) with plasma derived HBsAg particles (as used for immunization) (100 [Fl] µl of 1 [Fg] µg/ml in 0.05 M sodium carbonate-bicarbonate buffer, pH 9.6) were incubated with the plasma for 1 hr at 37 [E] ° C. Captured antibodies were then detected with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, IgG1 or IgG2a (1:4000 in PBS-Tween, 10% PBS: 100 [Fl] µl/well; Southern Biotechnology Inc., Birmingham, AL), followed by addition of o-phenylenediamine dihydrochloride solution (OPD, Sigma), 100 [Fl] µl/well, for 30 min at RT in the dark. The reaction was stopped by the addition of 4 N $H_2SO_4$, 50 [Fl] µl/well.

End-point dilution titers were defined as the highest plasma dilution that resulted in an absorbance value (OD 450) two times greater than that of non-immune plasma, with a cut-off value of 0.05. Anti-HBs titers of responding mice (endpoint titers > 10) were expressed as means SEM of individual animal values, which were themselves the average of triplicate assays.

*Mucosal humoral response*: This was carried out on fecal supernatants or recovered lung washes as for plasma (above) except samples were incubated on coated plates for 2 hr at 37°C and captured antibodies were detected with HRP-conjugated goat anti-mouse IgA (1:1000 in PBS-Tween. 10% PBS: 100 [Fl] µl/well; Southern Biotechnology Inc). Non-immune fecal pellet or lung wash solutions were used to determine negative control values. For lung wash solutions, anti-HBs endpoint dilution titers were reported (as described above), whereas for fecal pellet solutions, absorbance values (OD 450) greater than that of non-immune fecal pellet solution were calculated and expressed as mean SEM of individual OD 450 values, which were themselves the average of triplicate assays.

*Evaluation of CTL responses*: Spleens were removed from mice 4 wk after primary immunization or boost. *In vitro* assay of HBsAg-specific cytolytic activity was carried out as previously described (Davis *et al.*, 1998). In brief, single cell suspensions were prepared and suspended in tissue culture medium (RPMI 1640, 10 % FBS, Life Technologies, Grand Island, NY, supplemented with penicillin-streptomycin solution, 1000 U/ml, 1mg/ml final concentrations respectively, Sigma). Splenocytes (3 x $10^7$) were co-cultured for 5 days (37[E] ° C, 5% CO2) with 1.5 x $10^6$ syngeneic HBsAg-expressing stimulator cells (P815-preS, generously provided by F. V. Chisari, Scripps Institute, La Jolla, CA) that had been previously inactivated by irradiation (20 000 rad). Effector cells were harvested, washed, serially diluted and cultured with 5 x $10^4$ $^{51}$Cr-labeled HBsAg-expressing target cells (P815S) in round bottom 96-well culture plates (37[E] ° C, 5% CO2, 4 hr). Supernatant (100 [Fl] µl) was removed for radiation (gamma) counting. Spontaneous release was determined by incubating target cells without effector cells and total release by addition of 100 [Fl] µl 2 N HCl to the target cells. The percent lysis was calculated as [(experimental release - spontaneous release)/(total release - spontaneous release)] x 100. The percent specific lysis was calculated as % lysis with P815S - % lysis with P815 cells. CTL activity for responding mice [% specific lysis > 10 at effector:target (E:T) of 25:1] were expressed as mean SEM of individual animal values, which were themselves the average of triplicate assays.

Please re-write the entire set of pending claims as follows:

1. (Twice Amended) A method for inducing a mucosal immune response, comprising: administering to a mucosal surface of a subject in need of a mucosal immune response an effective amount for inducing a mucosal immune response of an oligonucleotide at least 8 nucleotides in length, having a sequence including at least the following formula:

5' $X_1$ $X_2$CG$X_3$ $X_4$ 3'

wherein C is unmethylated, wherein $X_1$, $X_2$, $X_3$, and $X_4$ are nucleotides, and

exposing the subject to an antigen to induce the mucosal immune response, and wherein the antigen is not encoded in a nucleic acid vector.

2. The method of claim 1, wherein the subject is actively exposed to the antigen.

3. The method of claim 2, wherein the antigen is delivered to a mucosal surface.

4. The method of claim 2, wherein the antigen is administered concurrently with the oligonucleotide.

5. The method of claim 2, wherein the antigen is delivered in conjunction with a colloidal dispersion system.

6. The method of claim 5, wherein the colloidal dispersion system is selected from the group consisting of macromolecular complexes, nanocapsules, microspheres, beads, and lipid-based systems.

7. The method of claim 6, wherein the lipid-based system is selected from the group consisting of oil-in-water emulsions, micelles, mixed micelles, and liposomes.

8. The method of claim 2, further comprising the step of administering a non-oligonucleotide mucosal adjuvant in conjunction with the antigen.

9. The method of claim 8, wherein the non-oligonucleotide mucosal adjuvant is selected from the group consisting of cholera toxin, derivatives of cholera toxin, labile toxin, derivatives of labile toxin, alum, MLP, MDP, saponins such as QS21, cytokines, oil-in-water and other emulsion formulations such as MF59, SAF, Montanide ISA 720 and PROVAX, PCPP polymers, and ISCOMS.

10. The method of claim 1, wherein the subject is passively exposed to the antigen.

11. The method of claim 10, wherein the subject is a subject at risk of developing an allergic reaction.

12. The method of claim 10, wherein the subject is a subject at risk of developing an infectious disease.

13. The method of claim 11, wherein the subject is at risk of developing cancer.

14. The method of claim 1, wherein the oligonucleotide is 8 to 100 nucleotides in length.

15. The method of claim 1, wherein the oligonucleotide includes a phosphate backbone modification which is a phosphorothioate or phosphorodithioate modification.

16. The method of claim 15, wherein the phosphate backbone modification occurs at the 5' end of the oligonucleotide.

17. The method of claim 15, wherein the phosphate backbone modification occurs at the 3' end of the oligonucleotide.

18. The method of claim 1, wherein $X_1X_2$ are nucleotides selected from the group consisting of: GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT, and TpG; and $X_3X_4$ are nucleotides selected from the group consisting of: TpT, CpT, ApT, TpG, ApG, CpG, TpC, ApC, CpC, TpA, ApA, and CpA.

19. The method of claim 1, wherein the oligonucleotide has a sequence including at least the following formula:

5' $TCNTX_1X_2CGX_3X_4$ 3'

wherein $X_1$, $X_2$, $X_3$,, and $X_4$ are nucleotides, N is a nucleic acid sequence composed of from about 0-25 nucleotides.

20. (Amended) The method of claim 1, wherein the antigen is selected from the group consisting of cells, cell extracts, proteins, polypeptides, peptides, polysaccharides, polysaccharide conjugates, peptide mimics of polysaccharides, lipids, glycolipids, carbohydrates, allergens, viruses and viral extracts and [muticellular organisms such as] parasites.

21. The method of claim 1, wherein the antigen is an allergen.

22. The method of claim 1, wherein the antigen is derived from an infectious organism selected from the group consisting of infectious bacteria, infectious viruses, infectious parasites, and infectious fungi.

23. The method of claim 1, wherein the subject is an asthmatic.

24. The method of claim 1, further comprising administering a cytokine to the subject.

25. The method of claim 1, further comprising administering a B-7 costimulatory molecule.

26. The method of claim 1, wherein the mucosal immunity is induced in a remote site.

27. (Amended) The method of claim 1, further comprising administering a boost of the oligonucleotide.

28. The method of claim 8, further comprising administering a boost of the oligonucleotide and the non-oligonucleotide mucosal adjuvant.

125. (New) The method of claim 1, wherein oligonucleotide is administered to a mucosal surface different from that at which the subject is exposed to the antigen.

126. (New) The method of claim 1, wherein the oligonucleotide is administered by inhalation.

128. (New) The method of claim 1, wherein the subject is exposed to the antigen by inhalation.

128. (New) The method of claim 1, wherein the oligonucleotide is formulated for ocular administration, rectal administration, vaginal administration, intranasal administration or inhalation.

129. (New) The method of claim 1, further comprising identifying a subject in need of a mucosal immune response.

130. (New) A method for inducing a mucosal immune response, comprising:

administering to a mucosal surface of a subject an effective amount for inducing a mucosal immune response of an oligonucleotide at least 8 nucleotides in length, formulated for ocular administration, rectal

administration, vaginal administration, intranasal administration or inhalation, and having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein $X_1$, $X_2$, $X_3$, and $X_4$ are nucleotides, and

exposing the subject to an antigen to induce the mucosal immune response, and wherein the antigen is not encoded in a nucleic acid vector.

Appendix B

1: Vaccine 1993 Sep;11(12):1179-84

# Cholera toxin and cholera B subunit as oral-mucosal adjuvant and antigen vector systems.

Holmgren J, Lycke N, Czerkinsky C.

Department of Medical Microbiology and Immunology, University of Goteborg, Sweden.

Cholera toxin (CT) and the analogous heat-labile enterotoxin (LT) from Escherichia coli have several immunomodulating effects which alone or in combination might explain their strong adjuvant action in stimulating mucosal IgA and other immune responses to admixed unrelated antigens after oral immunization. These effects include, depending on animal species and experimental systems, enhanced antigen presentation by a variety of cell types; promotion of isotype differentiation in B cells leading to increased IgA formation; and complex stimulatory as well as inhibitory effects on T-cell proliferation and lymphokine production. This adjuvant activity appears to be closely linked to the ADP-ribosylating action of CT and LT associated with enhanced cyclic AMP formation in the affected cells, and thus it may prove difficult to eliminate the enterotoxic activity without loss of adjuvanticity. However, through a separate mechanism, as an antigen-carrier system providing specific binding to epithelium including the M cells of intestinal Peyer's patches, both CT and its non-toxic binding subunit moiety (CTB) have been shown to markedly enhance the mucosal immune response to various foreign antigens or epitopes covalently linked to these molecules. This gives promise for the future use of CTB or related non-toxic binding derivatives as vehicles to facilitate induction of mucosal immune responses to a broad range of antigens for human vaccination purposes.

Publication Types:
Review
Review, Tutorial

PMID: 8256498 [PubMed - indexed for MEDLINE]

1: Vaccine 1999 Jan;17(1):19-25

# CpG motifs as immune adjuvants.

Klinman DM, Barnhart KM, Conover J.

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Bacterial DNA contains immunostimulatory motifs that trigger an innate immune response characterized by the production of predominantly Th1-type cytokines. These motifs consist of an unmethylated CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines. We examined whether synthetic oligodeoxynucleotides (oligos) expressing these motifs would act as adjuvants to boost the immune response to DNA- and protein-based immunogens. In vivo experiments demonstrate that CpG-containing oligos augment antigen-specific serum antibody levels by up to tenfold, and IFNgamma production by up to sixfold. These effects were optimized by physically linking the CpG-containing motifs to the immunogen.

PMID: 10078603 [PubMed - indexed for MEDLINE]

Appendix C



**ATTORNEY'S DOCKET NO. C1040/7006 (HCL/MAT)**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): McCluskie et al.
Serial No.: 09/316,199
Filed: May 21, 1999
For: METHODS AND PRODUCTS FOR INDUCING MUCOSAL IMMUNITY
Examiner: D. Nguyen
Art Unit: 1633

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## DECLARATION OF HEATHER L. DAVIS UNDER 37 C.F.R. §1.131

I, Heather L. Davis, state and declare the following:

1. I am a co-inventor of the above-identified patent application. I make this Declaration in support of an Amendment filed in connection with the above-identified patent application.

2. The idea that mucosal administration of a CpG oligonucleotide could induce a mucosal immune response to an antigen upon antigen exposure was conceived and reduced to practice in my laboratory prior to April 2, 1998, the effective filing date of U.S. Patent 6,218,371, issued April 17, 2001 to Krieg et al.

3. Corroborative support for the statement in ¶2, above, can be provided to the Examiner if this should be necessary.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Date

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